

Symposium Antibiotic Resistance StAR

MDROs: molecular diagnostic tools

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In this short lecture

MDROs:

focus on Gram-negatives, especially CPE

Molecular:

detection of antimicrobial resistance genes/proteins

No colorimetric/ biochemical tests (e.g., NP tests)

No rapid ASTs (e.g., Accelerate Pheno System)

Diagnostic tools:

those "rapid", commercially available, and used in the clinical context for

Colonization (gut)

Confirmation (colonies)

Bacteremia

No metagenomics (NGS)

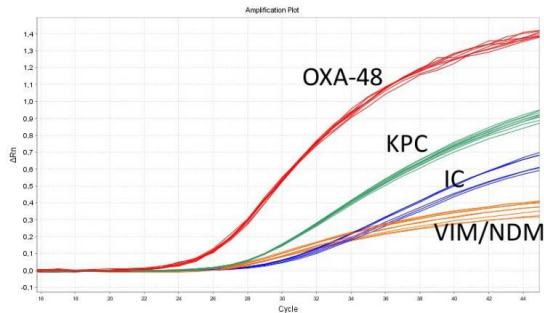
Check-Direct Screening (Check-Points)

Rectal
swab



- Real-time multiplex PCR
- Rapid preparation
- Time to results (<3 hrs)

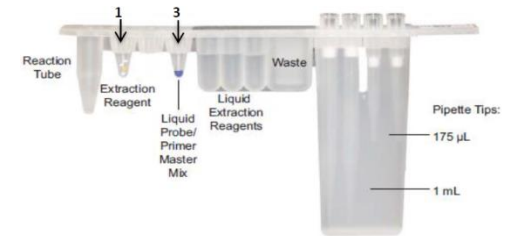
~30-50 Euro



BD MAX



Reagent strip



Check-Direct **ESBL**
Screen for BD MAX™

CTX-M-1 group
CTX-M-2 group
CTX-M-9 group
SHV-ESBLs

Check-Direct **CPE /CPO**
Screen for BD MAX™

KPC
OXA-48-like incl. OXA-181, OXA-232, OXA-244
VIM
NDM

GeneXpert (Cepheid)



- Add aliquot to elution, vortex, transfer to port S
- Insert cartridge to station (overall, **1 min**)
- Run time (**<1 h**)

~50 Euro

Real-time multiplex PCR

- Smart fluidic system
- Filtering and Sonication (DNA)
- Fluorescent-labeled hybr. probes (6 colors)

Xpert® MRSA/SA BC

Xpert® vanA/vanB

Xpert® Carba-R

KPC

NDM

OXA-48-like incl. OXA-181, OXA-232, OXA-244

VIM

IMP-1

Evaluation of a New Commercial Microarray Platform for the Simultaneous Detection of β -Lactamase and *mcr-1* and *mcr-2* Genes in *Enterobacteriaceae*

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New CT103XL (Check-Points)

Broad-spectrum: TEM and SHV

ESBLs: TEM, SHV, CTX-M, BEL, PER, GES, VEB

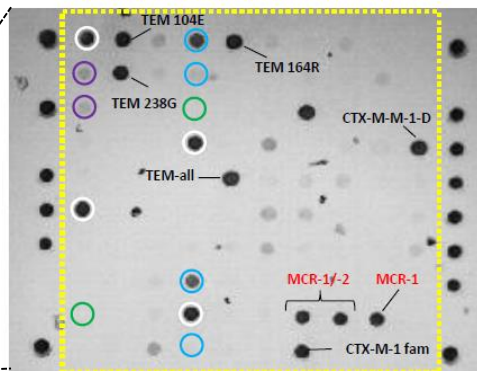
pAmpCs: CMY, DHA, FOX, ACC-1, ACT/MIR

Carba: KPC, NDM, VIM, IMP, GIM, SPM, OXA-48-like incl. 181/-232/-244, -23, -24, -58

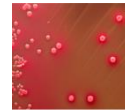
Mcr-1 and mcr-2



E. coli strain



Sensitivity and Specificity for all target genes: both ~100%



Confirmatory
test after ASTs



≥ 8 hrs

85 Euro



Rapid WGS



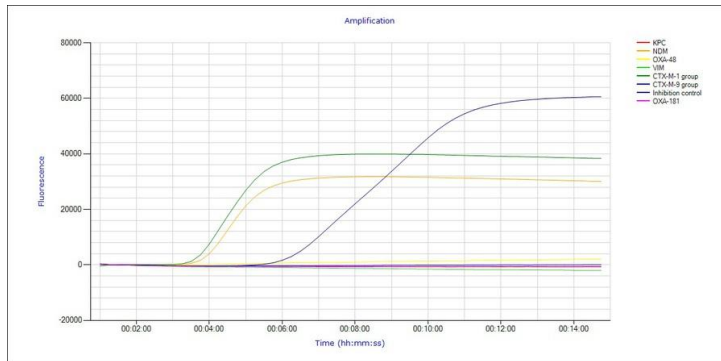
In few hrs
enough reads/coverage
to reports all ARGs

6 strains in 1 cell
~80 CHF each

Eazyplex (Amplex Diagnostics)



- Preparation (**5 min**)
- No DNA extraction/purification
- Run time (**<30 min**)



LAMP

Loop-mediated isothermal Amplification

Real-time fluorescent measurement

50 CHF

eazyplex® MRSA

S. aureus
mecA
mecC
S. epidermidis

eazyplex® VRE

eazyplex® SuperBug mcr-1

eazyplex® SuperBug Acineto

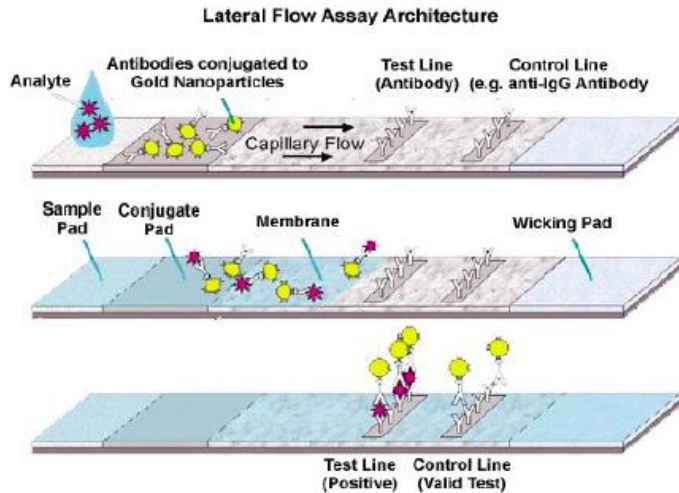
eazyplex® SuperBug complete

	SuperBug complete A	SuperBug complete B	SuperBug complete C
NDM	X	X	X
VIM	X	X	X
KPC	X	X	X
OXA-48	X	X	X
OXA-23	X	X	
OXA-40	X	X	
OXA-58	x		
OXA-181		x	X
IMP			x

eazyplex® SuperBug CRE

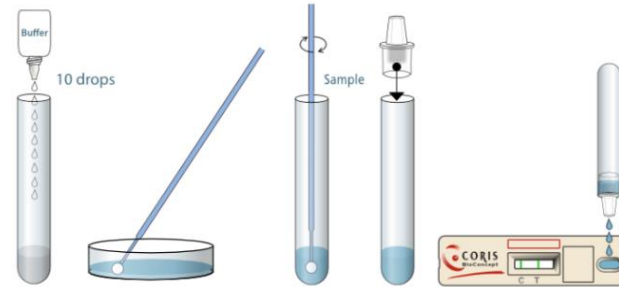
KPC,
NDM,
OXA-48^{incl. OXA-244}
and OXA 181,
VIM, as well as
CTX-M-1 and
CTX-M-9 group

Lateral Flow Assays / Immunochromatographic Tests

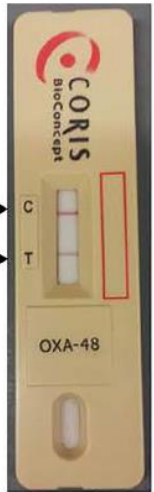


2×10^6 CFU/mL
(colonies or blood culture)

Within
15 min



Control → C
OXA-48 → T



Journal of Clinical Microbiology
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TARGET	PRODUCT NAME
OXA-48	OXA-48 K-SeT
KPC	KPC K-SeT
OXA-48 & OXA-163 & KPC	RESIST-3 O.O.K. K-SeT
OXA-48 & KPC & NDM	RESIST-3 O.K.N. K-SeT
OXA-23	OXA-23 K-SeT
OXA-48 & KPC NDM & VIM	RESIST-4 O.K.N.V.
OXA-48 & OXA-163 & KPC NDM & VIM	RESIST-5 O.O.K.N.V.
IMP	IMP K-SeT

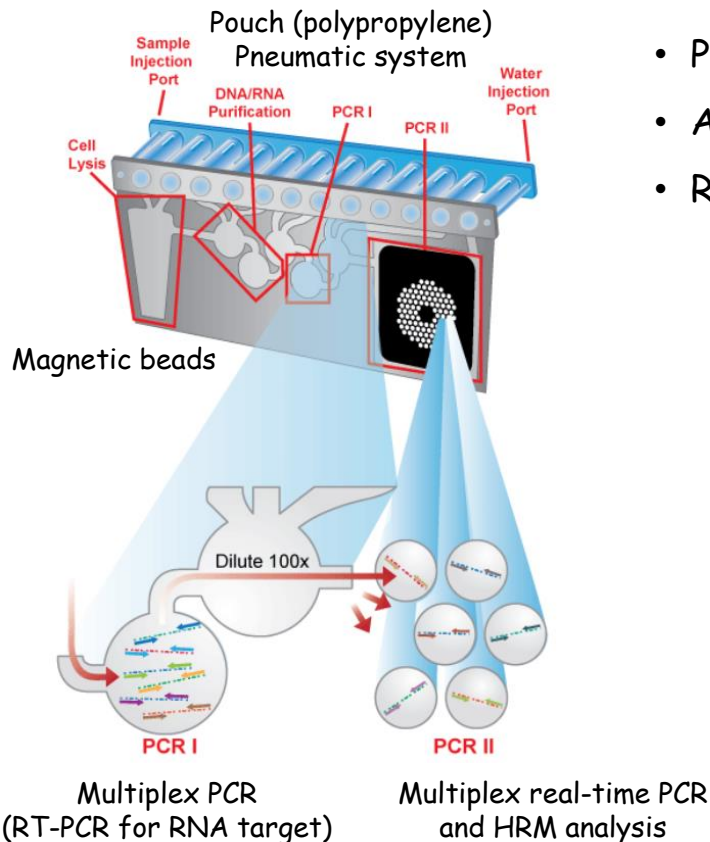
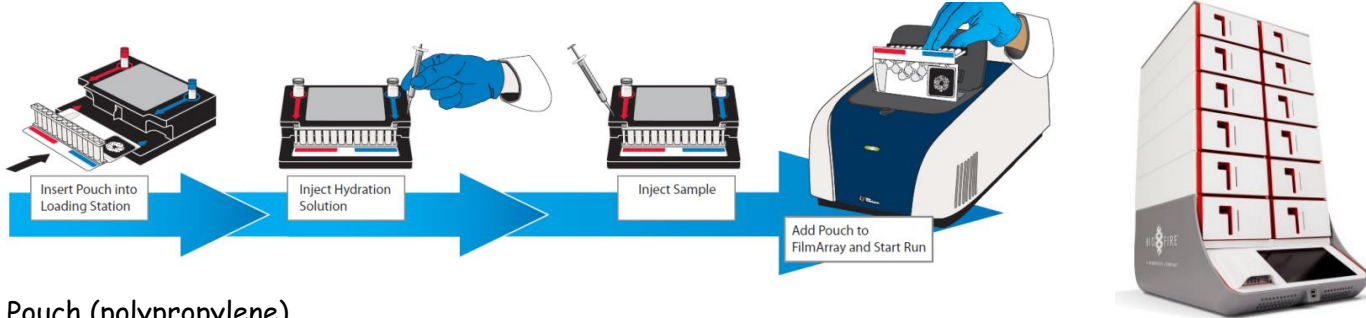
15 Euro

Youri Glupczynski*, Stéphanie Evrard, Te-Din Huang and Pierre Bogaerts
J Antimicrob Chemother 2019; **74**: 1284–1287



Target	n	sensitivity (95% CI)	specificity (95% CI)
Global performance of the RESIST-4 K-SeT assay for the collection of retrospective and prospective clinical isolates (n = 479)			
OXA-48-like incl. OXA-181, OXA-232, OXA-244	112	100 (95.9–100)	100 (98.8–100)
VIM	104	99 (94.0–99.9)	100 (98.7–100)
NDM	61	100 (92.6–100)	100 (98.9–100)
KPC	31	100 (86.3–100)	100 (99.0–100)
Other carbapenemases/non-carbapenemases	184		
Target	n	positive predictive value (95% CI)	negative predictive value (95% CI)
Positive and negative predictive values of the RESIST-4 K-SeT assay for the collection of prospective clinical isolates (n = 345)			
OXA-48-like incl. OXA-181	90	100 (94.9–100)	100 (98.2–100)
VIM	65	100 (93.4–100)	100 (98.3–100)
NDM	22	100 (81.5–100)	100 (98.5–100)
KPC	19	100 (79.1–100)	100 (98.5–100)
Other carbapenemases/non-carbapenemases	151		

BioFire FilmArray (bioMérieux)



- Preparation of the pouch
- Add pouch to FilmArray station (overall, **2 min**)
- Run time of about **1 h**

~100 Euro

The BioFire® FilmArray® Blood Culture Identification (BCID) Panel

GRAM-NEGATIVE BACTERIA:

- *Acinetobacter baumannii*
- *Haemophilus influenzae*
- *Neisseria meningitidis*
- *Pseudomonas aeruginosa*
- Enterobacteriaceae
- Enterobacter cloacae complex
- *Escherichia coli*
- *Klebsiella oxytoca*
- *Klebsiella pneumoniae*
- *Proteus*
- *Serratia marcescens*

GRAM-POSITIVE BACTERIA:

- *Enterococcus*
- *Listeria monocytogenes*
- *Staphylococcus*
- *Staphylococcus aureus*
- *Streptococcus*
- *Streptococcus agalactiae*
- *Streptococcus pneumoniae*
- *Streptococcus pyogenes*

ANTIMICROBIAL RESISTANCE GENES:

- *mecA* – methicillin resistance
- *vanA/B* – vancomycin resistance
- *KPC* – carbapenem resistance

YEAST:

- *Candida albicans*
- *Candida glabrata*
- *Candida krusei*
- *Candida parapsilosis*
- *Candida tropicalis*

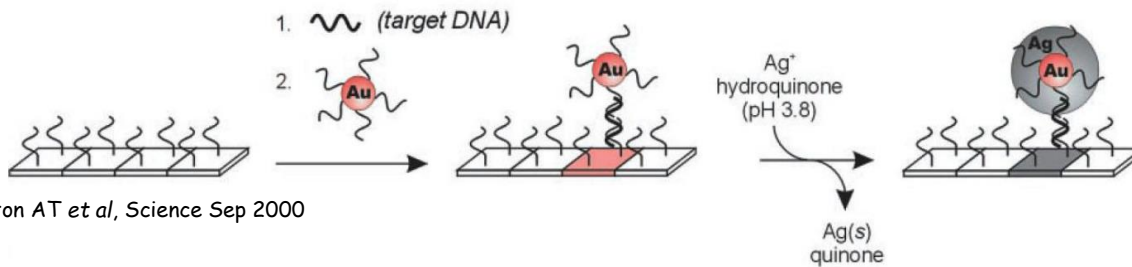
Verigene System (Luminex)



!



- Load cartridge, consumables, and sample (**5 min**)
- Automated sample preparation and processing
- Place slide from cartridge in reader (**2,5 hrs**)



Taton AT et al, Science Sep 2000

Microarray approach by using Au-nanoprobe as reporter and silver reduction to enhance signal

Gram-negatives cartridge

Species	Genus	Resistance
<i>Escherichia coli</i> *	<i>Acinetobacter</i> spp.	CTX-M (ESBL)
<i>Klebsiella pneumoniae</i>	<i>Citrobacter</i> spp.	IMP (carbapenemase)
<i>Klebsiella oxytoca</i>	<i>Enterobacter</i> spp.	KPC (carbapenemase)
<i>Pseudomonas aeruginosa</i>	<i>Proteus</i> spp.	NDM (carbapenemase)
<i>Serratia marcescens</i>		incl. OXA-48 OXA (carbapenemase)
		VIM (carbapenemase)

Gram-positives cartridge

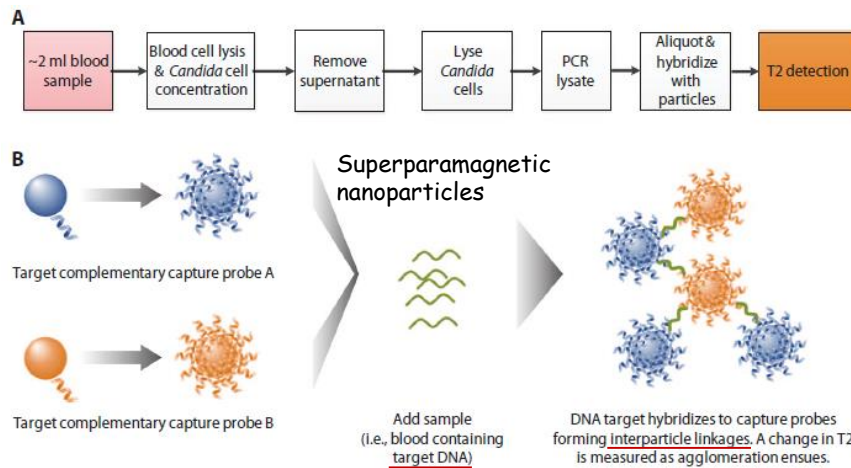
Species	Genus	Resistance
<i>Staphylococcus aureus</i>	<i>Staphylococcus</i> spp.	<i>mecA</i> (methicillin)
<i>Staphylococcus epidermidis</i>	<i>Streptococcus</i> spp.	<i>vanA</i> (vancomycin)
<i>Staphylococcus lugdunensis</i>	<i>Micrococcus</i> spp.	<i>vanB</i> (vancomycin)
<i>Streptococcus anginosus</i> Group	<i>Listeria</i> spp.	
<i>Streptococcus agalactiae</i>		
<i>Streptococcus pneumoniae</i>		
<i>Streptococcus pyogenes</i>		
<i>Enterococcus faecalis</i>		
<i>Enterococcus faecium</i>		

~50 Euro

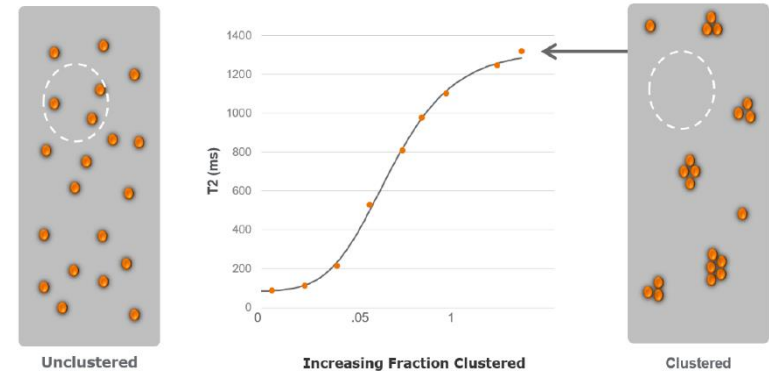
T2 Magnetic Resonance, T2MR (T2 Biosystems)



- Sample transferred to T2Dx (5 min)
- No extraction/purification
- Time to results (~3-5 ... max 9 hrs)
- Limit of detection: 1 CFU/mL



267 CHF



Available T2Bacteria Kit

- Gram-negatives: *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*
 - Gram-positives: *S. aureus*, *E. faecium*
- } ESKAPEc

THANK YOU!

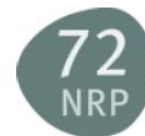
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